Electronic Supporting Information (ESI)

Multinuclear Ru(II) and Ir(III) decorated tetraphenylporphyrins as efficient PDT agents

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Contents:

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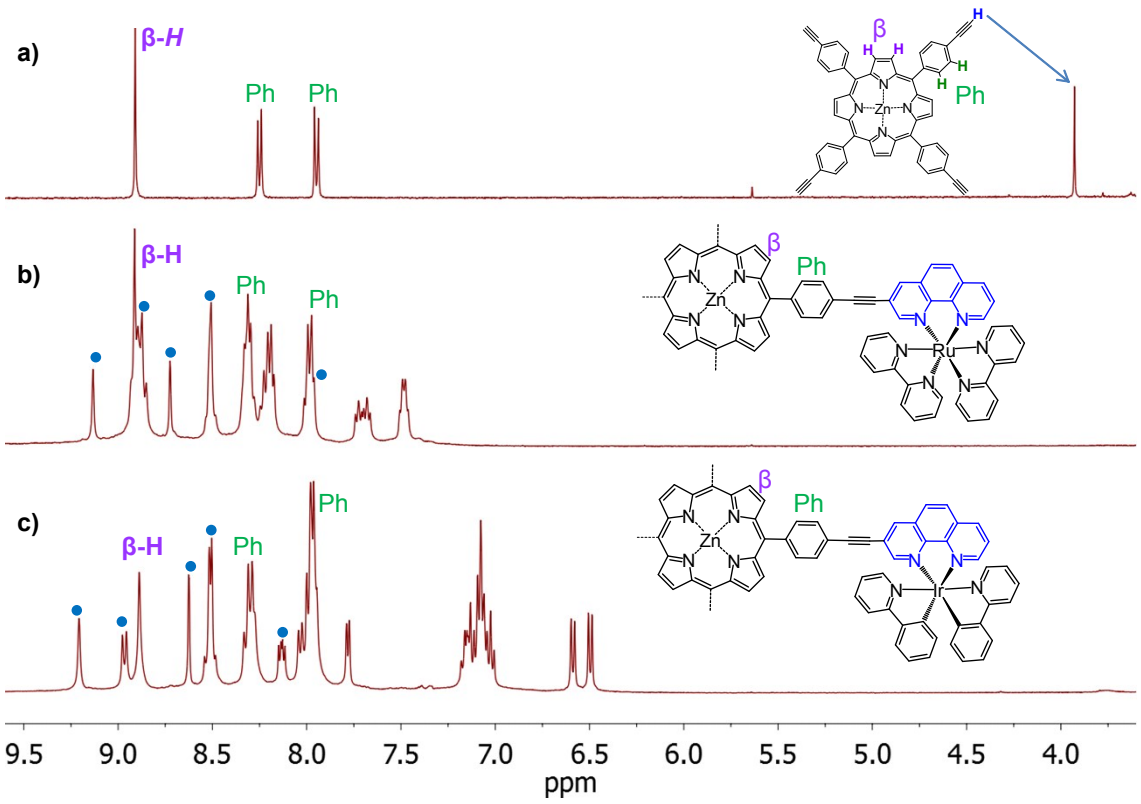

Fig. SChem1 ^{1}H NMR spectra in acetone-d_6 of a) **P-1**, b) **P-Ru** and c) **P-Ir**.

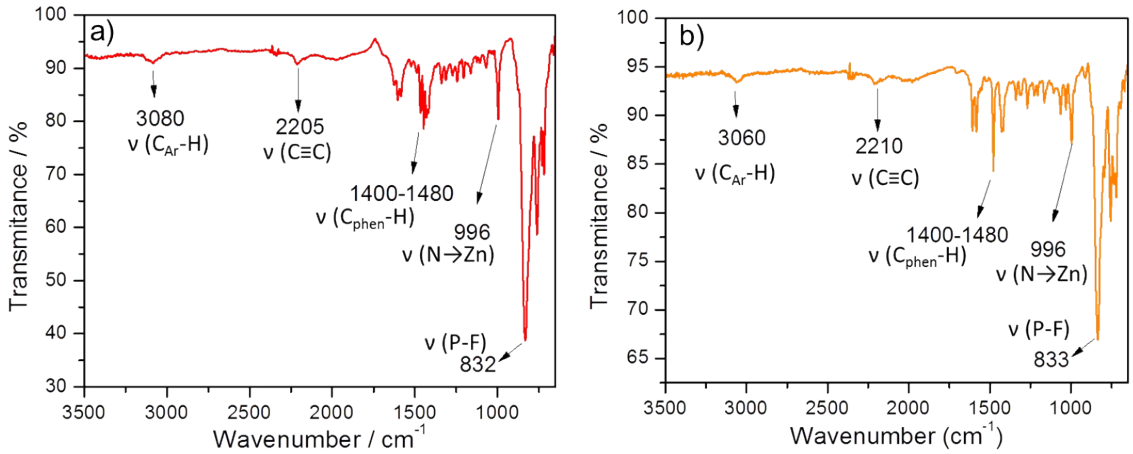

Fig. SChem2 FTIR-ATR spectra of a) **P-Ru** and b) **P-Ir**.

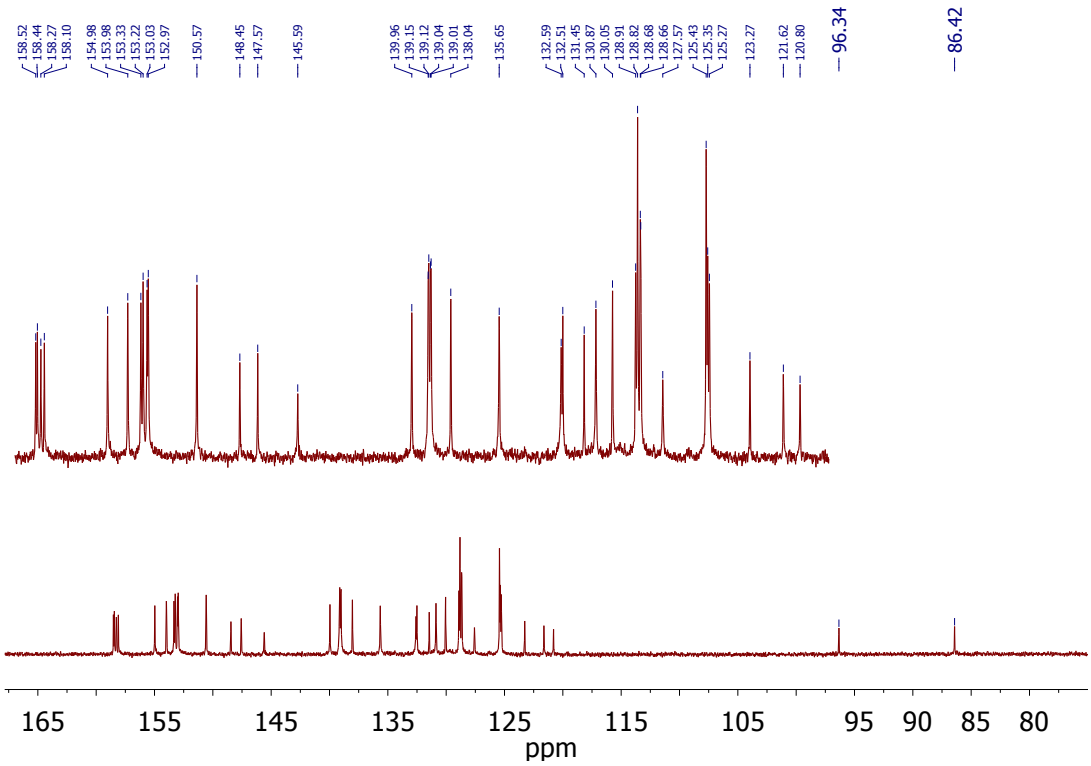

Fig. SChem3 $^{13}C\{^{1}H\}$ NMR spectra in acetone-d_6 of **P-Ru**.

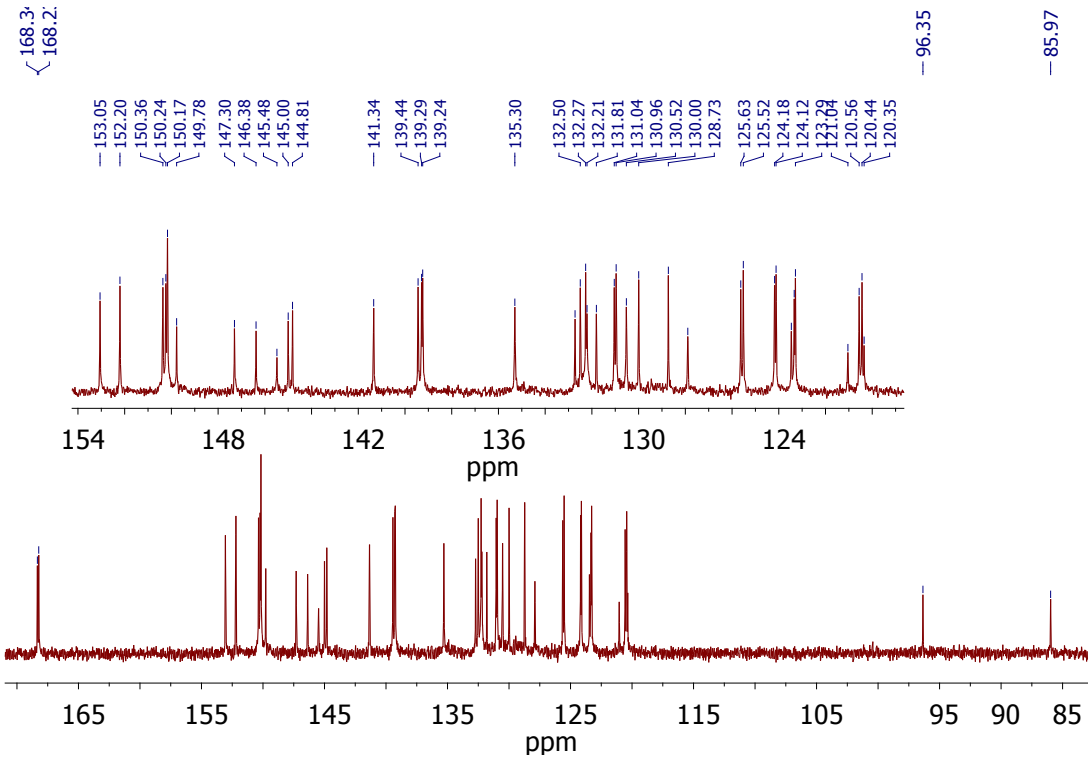

Fig. SChem4 $^{13}C\{^{1}H\}$ NMR spectra in acetone-d_6 of **P-Ir**.

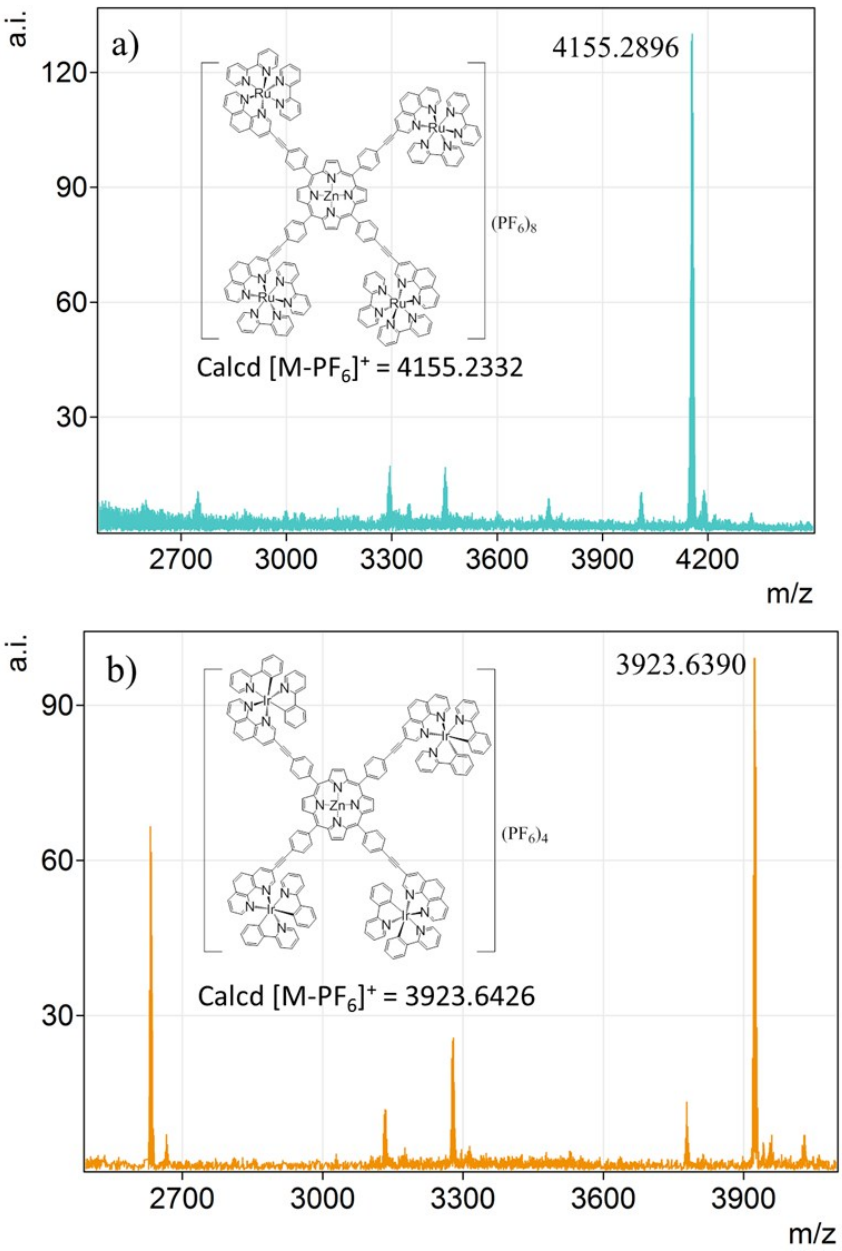

Fig. SChem5 MALDI-TOF HRMS of a) **P-Ru** and b) **P-Ir**.

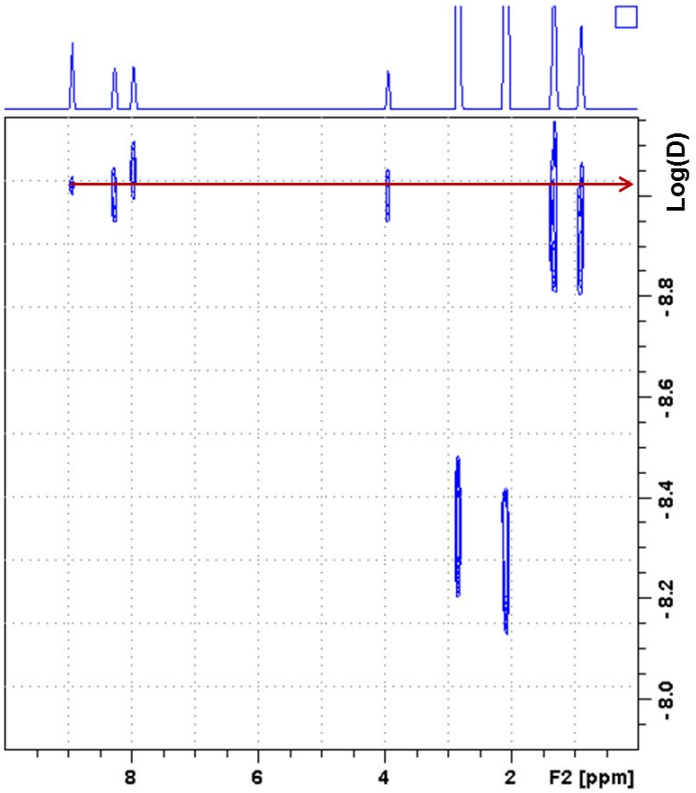

Fig. SChem6 2D DOSY spectrum of **P-1** in acetone-d_6 at 298K.

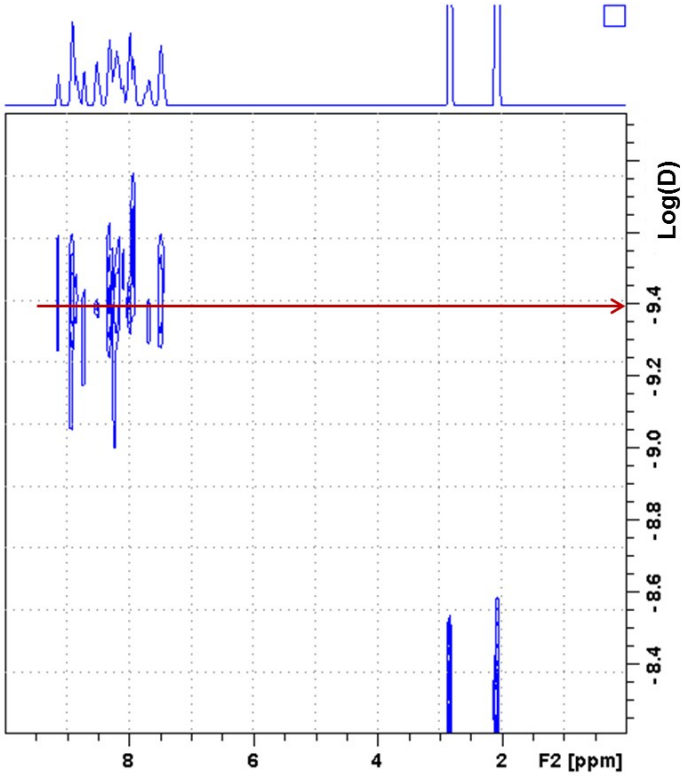

Fig. SChem7 2D DOSY spectrum of **P-Ru** in acetone-d_6 at 298K.

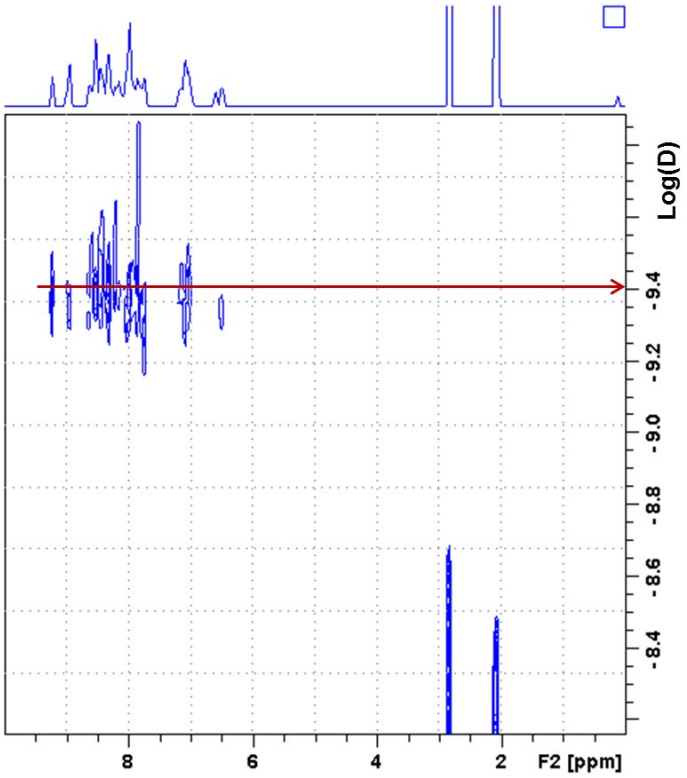

Fig. SChem8 2D DOSY spectrum of **P-Ir** in acetone-d_6 at 298K.

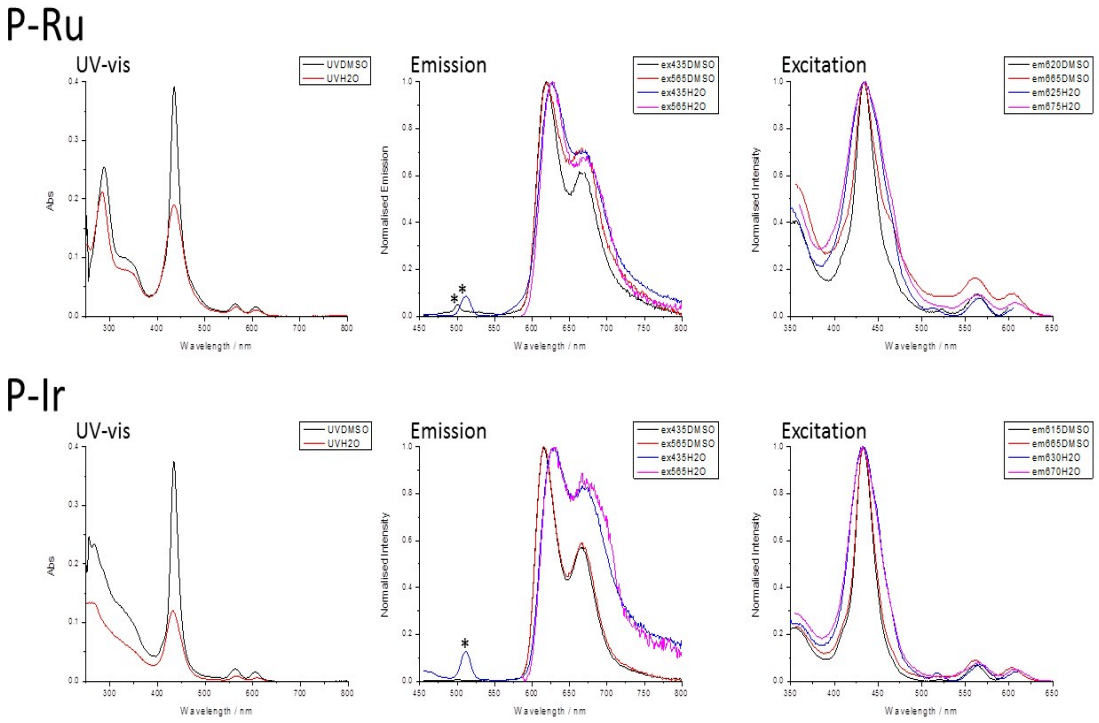

Fig. SPhoto1 UV/Vis absorption and emission data of (top) **P-Ru** and (bottom) **P-Ir** in H_20/DMSO solvent mixtures.

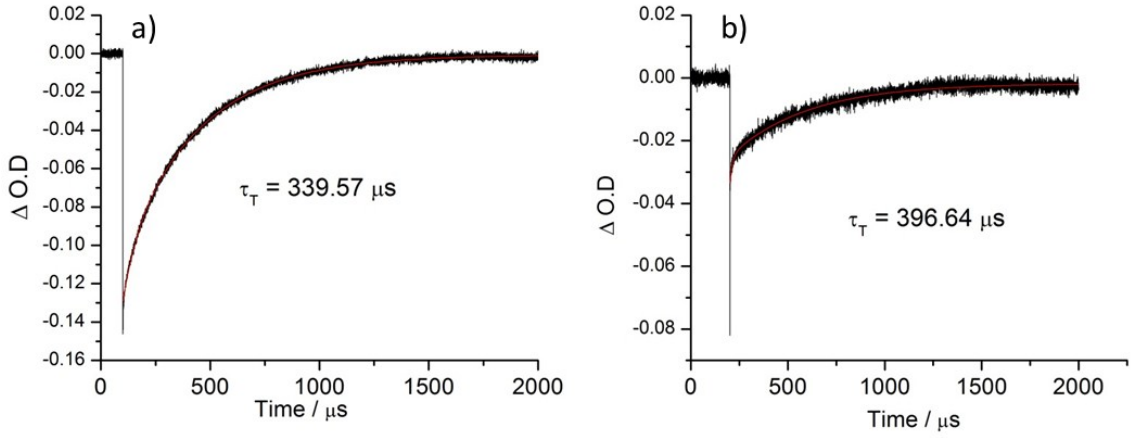

Fig. SPhoto2 Triplet lifetime decay traces of a) **P-Ru** (λ_{exc} = 450 nm, $\lambda_{detection}$ = 435 nm) and b) **P-Ir** (λ_{exc} = 410 nm, $\lambda_{detection}$ = 430 nm).

Additional Biological Experimental data:

Nuclear morphology

Nuclear changes were evaluated using Hoescht staining that specifically stains DNA. Nuclear morphology is related to different kind of cell deaths. Unaltered nuclei are usually oval and relatively big (green arrows). Necrotic nuclei present small round shape and are highly pyknotic (yellow arrows), while fragmented nuclei (red arrows) correspond to apoptotic cells.

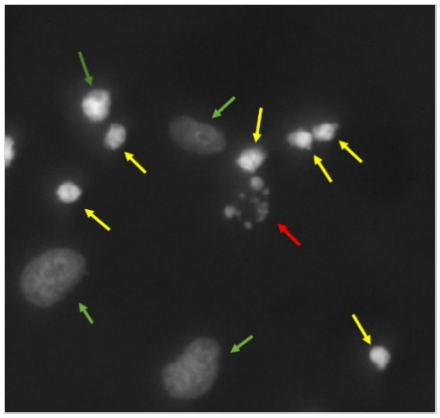

Fig. SBio1 Nuclear morphologies of SKBR3 cells incubated with **P-Ir** (0.5 μM) after irradiation. Three different morphologies were observed after Hoechst staining. Unaltered nuclei (green arrows) usually oval and relatively big; necrotic nuclei: small round shape and highly pyknotic nucleus (yellow arrows) and apoptotic nuclei: fragmented nuclei (red arrows).

Live Subcellular localization

Cells were seeded in 3.5 mm diameter glass bottom dishes (MatTek) at a density of 3 x 10^5 cells per dish. At 24 h after seeding, different concentrations of **P-Ru** or **P-Ir** (1 and 2 µM) were added. After 24 h incubation, the cells were washed three times with HBSS, maintained in fresh culture medium, and directly observed under a Confocal Laser Scanning Microscope (CLSM, Olympus XT7). The PS were ativated via excitation using a 405 nm laser of the CLSM for 1 min. The emission, between 600 and 700 nm, was used to observe the subcellular relocation of the PS after its activation.

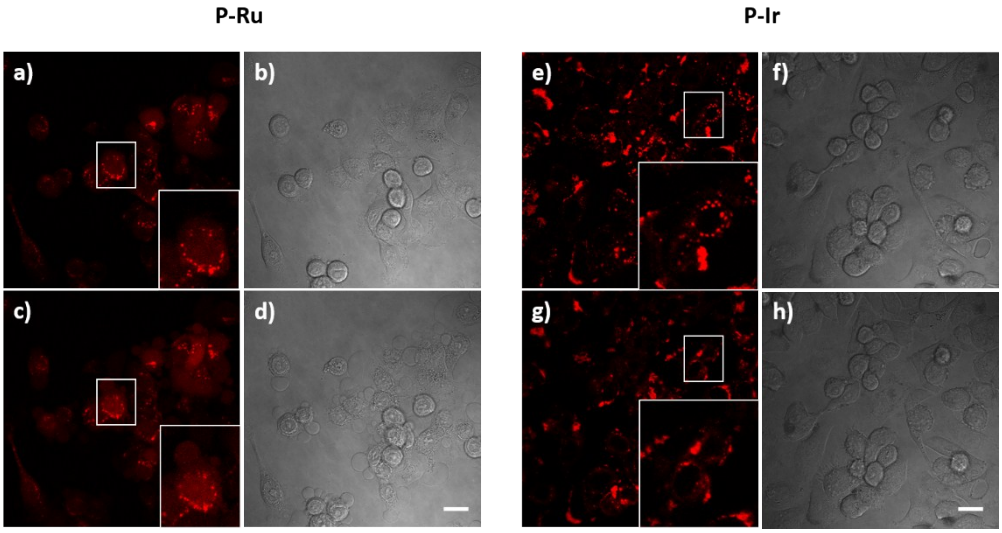

Fig SBio 2. Live SKBR-3 cells incubated with 2 µM **P-Ru** (a-d) or 1 µM **P-Ir** (e-h) for 24 h and observed under CLSM. To analyse the localisation of the PSs, bright field (b, d, f and h) or fluorescence mode was used (a, c, e and g). PS fluorescence emission was detected in the range of 600-700 nm by exciting the cells for a few seconds using the 405 nm laser (a, e). Then, the cells were excited for a minute using the same wavelength (405 nm) to evaluate the relocalisation of the PSs. Scale bar, 20 µm. Insets correspond to higher magnification of the framed cells.

Fixed cells subcellular localization

In order to confirm the lysosome localization of the PSs, we fixed the cell culture after it had been incubated with the PSs for 24 h. We performed an immunodetection of LAMP-1, a protein specific of the lysosome membrane. The cell membranes were stained with concanavalin A and the cell nuclei with Hoechst. Confocal 3D reconstruction allowed performing orthogonal projections of the z-stacks.

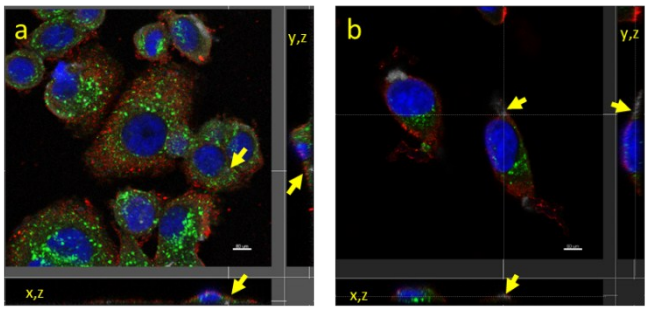

Fig. SBio3 Intracellular location of **P-Ru** (a) and **P-Ir** (b), analyzed by Confocal Laser Scanning Microscope (CLSM). Orthogonal projections of z-stacks of SKBR-3 cells incubated with 0.5 µM of PSs. a) **P-Ru** colocalize (yellow arrow) with LAMP-1 marker (x,z and x,y). b) **P-Ir** is adhered (yellow arrow) to the plasma membrane (x,z and x,y). Membrane is labelled with concanavalin A (red), lysosome marker LAMP-1 (green), nuclei (blue) and PSs (white).